sulted in a specific interaction with immobilized ATF-2 and pX, but not with the bacterial lysate BL21 that lacked these proteins (Fig. 3A).

CREB and ATF-related proteins mediate gene expression after activation by protein kinases (*14*). The identification of a protein kinase activity associated with pX (*15*) suggests that CREB or ATF-2 may be a substrate for the pX kinase. However, under conditions in which phosphorylation of calf thymus histone H1 by pX was demonstrable, protein kinase activity was not evident when CREB or ATF-2 was used as substrate (Fig. 3B). Consistent with these observations, we have detected the pX-mediated binding of truncated forms of both CREB and ATF-2 in which potential phosphorylation sites have been deleted (*9, 16*). Taken together, these data demonstrate that the direct protein-protein interaction resulting in DNA-binding specificity is independent of the kinase activity of pX.

The data presented demonstrate that pX engages a CRE-like sequence in the HBV enhancer by direct protein-protein interactions with CREB or ATF-2, resulting in an altered DNA binding specificity. Like other viral transactivators, such as VP16 and E1A (*7, 8*), pX does not bind DNA directly. However, pX differs from E1A and VP16 in that neither of these proteins alters the specificity of the DNA-protein interaction. The inability of CREB or ATF to bind to the HBV CRE-like element in the absence of pX may be due to the lack of complete homology to the CRE consensus sequence, the contribution from the flanking sequences, or both. Because a number of purified proteins bind to the HBV enhancer, pX may participate in the selection of specific protein combinations whose interactions result in the alteration of transcriptional activity. Whether pX possesses an independent activating domain as suggested (*17*) or augments functional domains present in the protein complex remains to be determined. Within the context of the HBV genome, it is possible that trans-activation occurs by alternative mechanisms that are not mutually exclusive, but that are dependent on the availability of certain transcription factors during various stages of the cell cycle or of hepatocyte differentiation. The establishment of complexes between pX and cellular proteins allows these proteins to bind to sequences for which the affinity of these cellular factors may be weak. A consequence of this type of interaction could be an expanded repertoire of cellular genes that become activated during viral infection.

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Inhibition of PDGF β Receptor Signal Transduction by Coexpression of a Truncated Receptor

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A mutated form of the platelet-derived growth factor (PDGF) β receptor lacking most of its cytoplasmic domain was tested for its ability to block wild-type PDGF receptor function. PDGF induced the formation of complexes consisting of wild-type and truncated receptors. Such complexes were defective in autophosphorylation. When truncated receptors were expressed in excess compared to wild-type receptors, stimulation by PDGF of receptor autophosphorylation, association of phosphatidylinositol-3 kinase with the receptor, and calcium mobilization were blocked. Thus, a truncated receptor can inactivate wild-type receptor function by forming ligand-dependent receptor complexes (probably heterodimers) that are incapable of mediating the early steps of signal transduction.

WHEN PDGF BINDS TO ITS SPECIFIC receptor on the cell surface, the receptor protein becomes phosphorylated on tyrosine residues (autophosphorylation), and the conformation of the cytoplasmic domain of the receptor is altered so that the receptor can interact with and phosphorylate cytoplasmic signaling molecules (*1–4*). In response to PDGF, the receptor forms noncovalently linked receptor dimers (*5, 6*). Whether dimerization is required for receptor autophosphorylation and for generating cytoplasmic signals is not known.

The wild-type β receptor for PDGF expressed in Chinese hamster ovary (CHO-K1) cells by transfection of cDNA (*7*) underwent a ligand-induced increase in density detected by analysis on sucrose gradients (Fig. 1A). This shift was consistent with the formation of receptor dimers (*5*). Although we cannot exclude the possibility that the shift in sedimentation of the receptor was due to association of the receptor with other nonreceptor molecules, this seems unlikely since we observed a similar shift (*8*) in a receptor mutant that lacked tyrosine kinase activity and was unable to associate with cytoplasmic molecules (*4, 9*). Only the receptor that sedimented at 11.5*S* reacted with

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antibody to phosphotyrosine (anti–P-Tyr) (*10*) (Fig. 1B). Little, if any, phosphotyrosine was detected in receptor that sedimented as a monomer (7.0*S*). These findings suggest that the receptor must form a complex (probably a dimer) in order to become autophosphorylated.

We next examined whether a truncated PDGF receptor that lacks most of its cytoplasmic domain but is still capable of binding PDGF (*11*) could form a complex with a wild-type receptor in intact cells. The truncated receptor was transfected into Balb/c 3T3 cells, which have native PDGF receptors, and a stable transfectant (3T3-Trunc) was selected (*7*). The cells were incubated with PDGF-BB (homodimeric form of PDGF-B chains), and lysates were immunoprecipitated with an antibody (Ab 88), which recognized an epitope from the wild-type receptor that was deleted in the truncated receptor (*12*). The truncated receptor was coimmunoprecipitated with the wild-type receptor only in lysates from PDGF-stimulated cells (120-kD band in Fig. 2). Addition of SDS to the lysis buffer disrupted the receptor complex, and the truncated receptor was no longer immunoprecipitated by Ab 88, which still recognized the wild-type receptor. Thus, the truncated receptor formed a noncovalent complex with wild-type receptor in a ligand-dependent manner. It is likely that this complex is a dimer (Fig. 3) although we cannot exclude the possibility that higher order oligomers form between wild-type and truncated receptors.

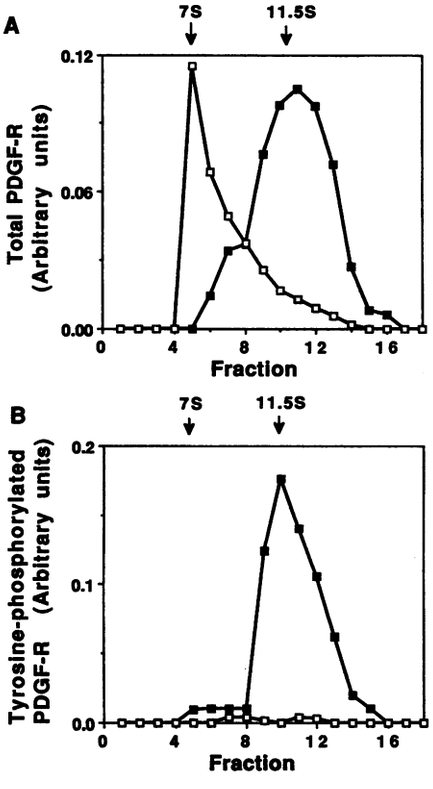


Fig. 1. Sucrose gradient sedimentation analysis of PDGF receptor in intact cells. CHO-K1 cells expressing wild-type PDGF receptors (*7*) were incubated at 4°C in the presence (closed squares) or absence (open squares) of PDGF-BB. Cell lysates were fractionated by ultracentrifugation on sucrose gradients (5 to 20%) (*25*). Each fraction was analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) (7%) and immunoblotted with antibody to the PDGF receptor (Ab 77) (**A**). The blots were probed again with anti–P-Tyr to detect phosphorylated receptor (**B**). Bound antibody was detected with ^{125}I-labeled protein A. The signals in the autoradiogram were quantified by densitometric scanning of the 180-kD receptor.

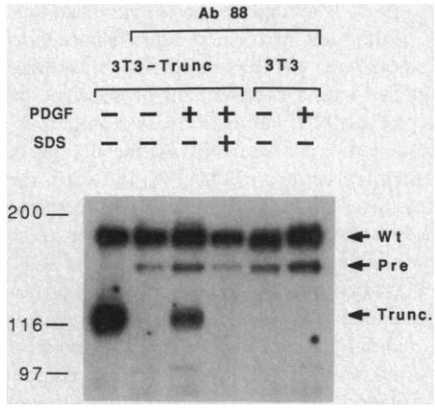


Fig. 2. PDGF-dependent formation of a complex between wild-type and truncated mutant receptors in intact cells. Cultures of Balb/c 3T3 fibroblast transfectants expressing the truncated PDGF receptors and native wild-type receptors (3T3-Trunc) or parental 3T3 cells (3T3) were incubated for 3 hours at 4°C in the presence or absence of PDGF-BB (2 nM) and then lysed with detergent. The lysates were immunoprecipitated with antibody to the receptor (Ab 88) (*26*). One lysate contained 0.1% SDS as indicated. Immunoprecipitates were fractionated by SDS-PAGE (6%), then transferred to nitrocellulose membranes and probed with another receptor antibody (Ab 77) that recognizes an extracellular sequence of the receptor (*12*). Wild-type receptor (180 kD), its precursor (160 kD), and truncated receptor (120 kD) proteins were visualized by autoradiography with ^{125}I-labeled protein A (indicated by arrows). The total surface expression of wild-type and truncated receptors was assessed (first lane) with lysate that was partially purified by wheat germ agglutinin (WGA)–Sepharose before the immunoblotting with Ab 77. The amount of lysate in the first lane was one fifth that used for other lanes. The film (Kodak XAR-5) was exposed for 24 hours at −70°C. Molecular size markers are in kilodaltons.

The apparent formation of a heterodimer between wild-type and truncated receptors was also investigated by cross-linking experiments (Fig. 3). When a covalent cross-linking agent was added to intact Balb/c 3T3 cells in the presence of PDGF, a 360-kD band that had the electrophoretic mobility expected of wild-type receptor dimer and a 180-kD band corresponding to receptor monomer were seen in immunoblots probed with antibody to the receptor (Fig. 3A). However, in 3T3-Trunc cells expressing both wild-type and truncated receptors, PDGF induced formation of two other species, one at 300 kD and one at 240 kD. The 300-kD species is probably a heterodimer of wild-type and truncated receptor. This species was not found in cells that had only wild-type receptor (Fig. 3, lane 3). In 3T3-Trunc cells the 300-kD species was recognized by an antibody to COOH-terminal sequences that are present in the wild-type receptor but deleted in the truncated receptor. The finding that this cross-linked 300-kD protein is present only in cells containing the truncated receptor and yet has sequences found only in the full-length receptor suggests that it represents a heterodimer of wild-type and truncated receptors. The 240-kD species is probably a homodimer of the 120-kD truncated receptor.

The 360-kD species (Fig. 3A) is probably a homodimer of the wild-type receptor, since it is present in both the wild-type cells and in 3T3-Trunc cells. However, we cannot rule out formation of a trimer (two 120-kD truncated receptors and one wild-type receptor) that might comigrate in the broad band in the 360- to 420-kD region of the gel (Fig. 3).

To determine whether the cross-linked complexes underwent PDGF-stimulated tyrosine phosphorylation, we used anti–P-Tyr to detect tyrosine-phosphorylated molecules in immunoblots (Fig. 3B). Only the bands corresponding to the wild-type homodimer (360 kD) and monomer (180 kD) became phosphorylated on tyrosine in response to PDGF. The tyrosine-phosphorylated 180-kD band (Fig. 3B) probably represents receptor dimer that was not cross-linked. The putative 300-kD heterodimer did not become phosphorylated. This result showed that the wild-type receptor is not capable of autophosphorylation when it is in a complex with truncated receptor and suggests that autophosphorylation is an intermolecular reaction between two wild-type receptors.

To test whether the truncated receptor can block transduction of signals by the wild-type receptor, we used a *Xenopus* oocyte expression system in which we could control the relative amounts of each receptor and achieve an excess of mutant receptors over the wild-type receptor. Receptor cDNAs were transcribed in vitro (*13*), and the mRNAs were microinjected into oocytes (*14*). When wild-type receptor mRNA was microinjected, proteins of 190 kD and 160 kD were expressed corresponding to mature and precursor forms of the receptor, respectively. The amounts of expression of wild-type and truncated receptor were comparable and were linear over a concentration range of 0 to 75 ng of injected receptor mRNA per oocyte.

We examined whether coexpression of an

excess of truncated receptors with wild-type receptors inhibited ligand-induced phosphorylation of the wild-type receptor. Oocytes were injected with a fixed amount of wild-type receptor mRNA and an increasing amount of truncated receptor mRNA. As the amount of truncated receptor mRNA was increased, the amount of phosphorylated wild-type receptors decreased, while expression of wild-type receptor was unchanged (Fig. 4). The loss of autophosphorylated wild-type receptor was probably due to the formation of heterodimers that could not undergo ligand-induced autophosphorylation (Fig. 3). If ligand-induced receptor pairing occurs without preference for truncated receptor or wild-type receptor, but only depends on the relative amounts of each receptor expressed on the cell surface, then the amounts of receptor homodimer and heterodimer can be predicted by a simple stochastic model (*15*). The measured loss in phosphorylated receptors was coincident with the loss of wild-type receptor homodimers predicted by the model (Fig. 4). The coincidence of the experimental data and the predicted curve also suggests that most, if not all, of the PDGF receptors on the cell surface form dimers in response to PDGF.

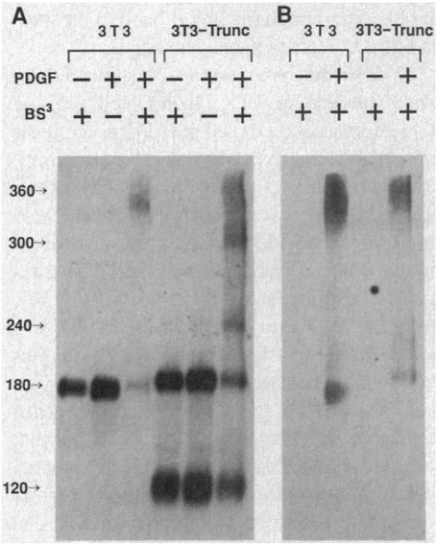
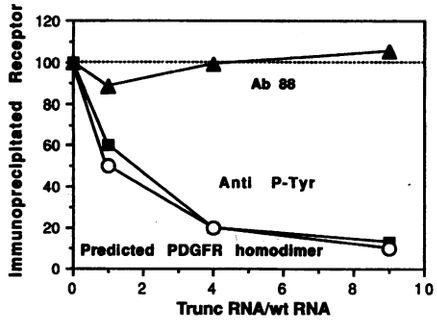


Fig. 3. Defective autophosphorylation of PDGF-induced heterodimers of wild-type and truncated receptors. Balb/c 3T3 cells (3T3) alone and Balb/c 3T3 cell transfectants that expressed both wild-type and truncated PDGF receptors (3T3-Trunc) were incubated in the absence or presence of PDGF-BB (3 nM) for 3 hours at 4°C, then exposed to the cross-linking agent, 3,3′-bis(sulfosuccinimidyl)suberate (BS^3, Pierce; 1 mM) for 20 min before detergent lysis (*26*). The lysates were partially purified by binding to WGA-Sepharose, fractionated by SDS-PAGE (4.5%), transferred to nitrocellulose membranes, and probed with Ab 77 (**A**) or anti–P-Tyr (**B**). Bound antibody was detected with ^{125}I-labeled protein A. The autoradiogram was exposed for 24 hours at −70°C. Arrows point to the receptor complexes described in the text. Anti–P-Tyr was slightly more sensitive in detecting phosphorylated receptor than Ab 77. Apparent molecular sizes (in kilodaltons) were determined by extrapolation of data with standards in the range of 96 to 200 kD.

Fig. 4. Progressive inhibition of wild-type receptor autophosphorylation by co-expression of increasing amounts of truncated receptor. Oocytes were coinjected with wild-type receptor mRNA (2 ng per oocyte) and various amounts of truncated receptor mRNA (0 to 18 ng per oocyte) and were then labeled with [^{35}S]methionine (*27*). Oocytes were stimulated with 1 nM PDGF-BB for 3 hours at 4°C before they were lysed with detergent. The lysates were analyzed by immunoprecipitation with either Ab 88 which recognized the total wild-type receptor pool (closed triangles) or with anti–P-Tyr which recognized only tyrosine-phosphorylated receptors (closed squares) (*26*). The immunoprecipitated receptors were analyzed by SDS-PAGE (6%), and the amount of receptor was quantified by autoradiography and densitometric scanning of the receptor band. The values were normalized relative to the signal from the oocytes injected with wild-type receptor mRNA alone and plotted as a function of the ratio of mutant receptor to wild-type receptor mRNA (closed symbols). The amounts of wild-type receptor homodimer predicted by the model (*15*) are also plotted (open circles).



We next examined the effects of truncated receptors on signal transduction by wild-type receptors. PDGF stimulates phosphatidylinositol (PI) hydrolysis and changes in Ca^{2+} flux (*16*). In oocytes, other receptors capable of hydrolyzing PI can increase Ca^{2+} efflux (*17, 18*). Therefore we used an assay of PDGF-stimulated Ca^{2+} efflux as an index of receptor activation and signal transduction (*19*). In response to PDGF-BB (1 nM), a large increase in Ca^{2+} efflux was observed in oocytes that expressed wild-type receptors (Table 1). The response was detected when PDGF concentrations as low as 0.01 nM were applied to the oocytes. The truncated receptor in oocytes did not evoke Ca^{2+} efflux in the presence of 1 nM PDGF (Table 1). Higher concentrations of PDGF (30 nM) were also ineffective. In oocytes that were coinjected with wild-type receptor mRNA and a 90-fold excess of truncated receptor mRNA, the Ca^{2+} efflux response to PDGF was completely abolished (Table 1). When a 90-fold excess of mRNA of the serotonin 1c receptor (*20*) or the basic fibroblast growth factor (bFGF) receptor (*21*) was coinjected with wild-type PDGF receptor mRNA, there was no effect on the Ca^{2+} efflux induced by PDGF (*22*).

The inhibitory effects of truncated receptors were overcome when the expression of wild-type PDGF receptors was increased by injecting more (20 ng) wild-type receptor mRNA (*22*). We also tested the effect of the PDGF receptor mutant on the function of the bFGF receptor. Three injections of a 90-fold excess of truncated PDGF receptor mRNA, along with wild-type bFGF receptor and wild-type PDGF receptor mRNA, completely eliminated PDGF-induced Ca^{2+} efflux. However, there was no effect on Ca^{2+} efflux induced by bFGF (Table 1). These observations confirmed that the mutant PDGF receptor specifically inhibited wild-type PDGF receptor function and had no effect on signal transduction by other receptors.

The inhibitory effect of mutant receptors depended on the amount of injected mRNA and on the amount of PDGF used for the assay. When a saturating concentration of

Table 1. Inhibitory effect of the truncated receptor on calcium efflux stimulated by wild-type PDGF receptor. Oocytes were injected with wild-type PDGF receptor (PDGFR) mRNA alone (0.4 ng per oocyte) or coinjected with wild-type receptor mRNA (0.4 ng) and truncated PDGF receptor mRNA (36 ng per oocyte) (PDGFR + Trunc). Some oocytes (different batch) were coinjected with wild-type PDGF receptor mRNA (0.2 ng per oocyte) and bFGF receptor mRNA (0.2 ng per oocyte) plus truncated PDGF receptor mRNA (18 ng per oocyte) (PDGFR + FGFR + Trunc). As a control, oocytes were coinjected with wild-type PDGF receptor and bFGF receptor mRNAs (0.2 ng per oocyte) but no truncated PDGF receptor mRNA (PDGFR + FGFR). Ca^{2+} efflux assay was performed as described (*19*). Mean values (10^2 cpm) of first 10 min Ca^{2+} efflux after addition of PDGF-BB (1 nM) or bFGF (10 ng/ml) are shown together with ± SD (n = 4); nd, not determined.

Ligand	Ca^{2+} efflux in oocytes injected with various mRNAs (10^2 cpm)				
	PDGFR	Trunc	PDGFR + Trunc	PDGFR + FGFR	PDGFR + FGFR + Trunc
None	6 ± 1	4 ± 1	4 ± 1	14 ± 1	5 ± 1
PDGF	234 ± 20	4 ± 1	4 ± 1	344 ± 40	8 ± 1
bFGF	nd	nd	nd	256 ± 16	254 ± 22

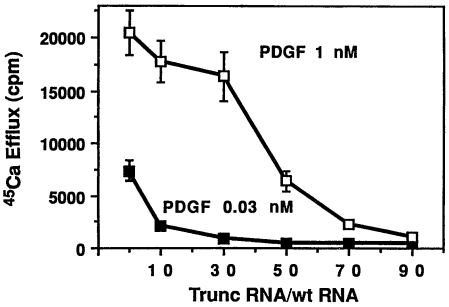


Fig. 5. Inhibitory effect of the truncated PDGF receptor at different ligand concentrations. Oocytes were coinjected with wild-type receptor mRNA (0.4 ng per oocyte) and various amounts of truncated receptor mRNA (0 to 36 ng per oocyte). Values for Ca^{2+} efflux measured 10 min after addition of either 1 nM PDGF-BB or 0.03 nM PDGF-BB are shown as mean values ± SD (n = 4). The abscissa indicates ratio of mutant receptor mRNA to wild-type receptor mRNA.

PDGF (1 nM) was used, a 70- to 90-fold excess of mutant mRNA was required to eliminate the function of the wild-type receptor. However, when a lower concentration of PDGF (0.03 nM) was used, a 10- to 30-fold excess of mutant receptor mRNA abolished the wild-type receptor response (Fig. 5).

The elimination of wild-type receptor function by mutant PDGF receptors was not due to suppression of wild-type receptor translation or alteration of protein processing. In oocytes coinjected with both the wild-type and truncated receptor (90-fold excess) mRNAs, the expression level of wild-type receptors was only marginally altered when compared to the amount of this protein expressed in oocytes injected with only wild-type receptor mRNA (*22*).

The inhibitory effect of the mutant receptors on wild-type PDGF receptor signal transduction was not restored by addition of 100 times more ligand (100 nM PDGF). Furthermore, when the PDGF-containing medium that did not induce a Ca^{2+} efflux in a group of oocytes coexpressing mutant and wild-type PDGF receptors was removed and transferred to a group of oocytes expressing only wild-type receptors, a full Ca^{2+} efflux response was evoked (*22*). These experiments exclude the possibility that the inhibitory effect of the mutant receptors could be attributed to depletion of the ligand from the medium.

Another PDGF-stimulated reaction that may be important in mediating the mitogenic effects of PDGF is the ligand-induced association of PI-3 kinase with the receptor (*2*). The truncated receptor blocked PDGF-stimulated PI-3 kinase association with the wild-type receptor in oocytes (*22*). Partial inhibition of association of PI-3 kinase with the receptor was observed even when equivalent amounts of truncated receptor mRNA and wild-type receptor mRNA were coinjected (*22*). On the average a two- to fourfold excess of mutant to wild-type mRNA was required to block half of the PI-3 kinase response (*22*). The lower amount of mutant receptor required to inhibit association of PI-3 kinase with the receptor (two- to fourfold excess) compared to the amount required to block half of the calcium efflux (~40-fold excess) suggests that the calcium efflux response is more sensitive to a given level of activated (tyrosine-phosphorylated) receptor than is the PI-3 kinase response. Thus a greater inhibition of receptor autophosphorylation is required to detect an effect on the calcium response.

There have been previous studies in which kinase-defective mutants of either insulin receptor or epidermal growth factor receptor inhibit function of their respective wild-type receptors or kinase-active mutant (*23, 24*). However, in these studies the mutant receptors did not inhibit the autophosphorylation of wild-type receptors and the mechanisms by which the kinase-defective mutants inhibited wild-type receptor function were not known. We have observed ligand-induced formation of inactive receptor complexes between wild-type receptor and mutant receptor. These complexes appear to be incapable of autophosphorylation and signal transduction.

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7. For expression in CHO-K1 or Balb/c 3T3 (clone A31) cells, the wild-type receptor and the truncated mutant cDNA were cloned into an expression vector, pSV7d, in which the cDNA was under the transcriptional control of the simian virus 40 early promoter. Cells were cotransfected with the expression plasmid together with pSV2 neo by the calcium phosphate method. Stable transfectants were selected in medium containing G418 (BRL/Gibco) and screened by immunoblotting with antibody to receptor (Ab 77) (*12*).
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10. The antibody to phosphotyrosine (4G10) was a murine monoclonal antibody raised against phosphotyramine (*4*).
11. The truncated mutant was obtained by deletion of murine β receptor for PDGF [Y. Yarden *et al.*, *Nature* **323**, 226 (1986)] at putative adenosine triphosphate–binding site, in which a single restriction site was created by site-directed mutagenesis. The mutant maintained a functional extracellular and transmembrane region but lost the kinase domain and putative autophosphorylation sites. The truncated receptor and wild-type receptor expressed in CHO cells (7) showed comparable affinity for ligand.
12. Two rabbit polyclonal PDGF β-receptor antibodies were used [M. T. Keating and L. T. Williams, *J. Biol. Chem.* **262**, 7932 (1987)]. Antibody 77 was directed against a synthetic peptide corresponding to amino acid residues 425 to 446 located in the fifth immunoglobulin-like domain, which is extracellular. A synthetic peptide containing amino acid residues 738 to 760 in the kinase insert region was used to generate an antibody specific for the cytoplasmic domain (Ab 88).
13. The 5.2-kb cDNA of the wild-type receptor and the 2.0-kb cDNA of the truncated receptor were inserted into pGEM-4Z (Promega Biotec.) at the 3′ side of an SP6 RNA polymerase promoter. In vitro transcripts (mRNAs) containing a 5′-GpppG cap (Pharmacia) were prepared from the linearized plasmid templates with Sp RNA polymerase [P. A. Krieg and D. A. Melton, *Methods Enzymol.* **155**, 397 (1987)].
14. Fully grown oocytes (Dumont stage VI) were obtained from *Xenopus laevis*. Oocytes were defolliculated either manually or by collagenase treatment (Sigma type II, 1 mg/ml) and maintained at 19°C in modified Barth's saline solution (MBS) with Hepes (15 mM, pH 7.6), bovine serum albumin (1 mg/ml), penicillin G (100 U/ml), and streptomycin (100 μg/ml). Oocytes were injected with 50 nl of RNA solution or pure water as described [A. Colman, in *Transcription and Translation: A Practical Approach*, B. D. Hames and S. J. Higgins, Eds. (IRL Press, Washington, DC, 1984), pp. 271–302.].
15. If the ratio of the concentration of mutant to wild-type receptor is x, then the probabilities of homo- and heterodimers forming under conditions in which all of the receptors exist as dimers at saturating concentration of ligand would be as follows: wild-type receptor homodimer, $1/(1 + x)^2$; heterodimer, $2x/(1 + x)^2$; mutant receptor homodimer, $x^2/(1 + x)^2$. The predicted amount of wild-type homodimer would be $1/(1 + x)$ times the amount of homodimer in the oocytes that have no mutant receptors expressed.
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19. The Ca^{2+} efflux from injected oocytes was quantitated by measuring $^{45}Ca^{2+}$ efflux (*17*). Oocytes incubated for 2 days after injection were treated with $^{45}Ca^{2+}$ (100 μCi/ml) in 0.5 ml of Ca^{2+}-free MBS for 3 hours at 19°C. After being washed with normal MBS, groups of eight oocytes were placed in 500 μl of MBS per well in a 24-well plastic cell culture plate. The medium was collected and replaced every 10 min, and the radioactivity in the medium was counted by a liquid scintillation counter. After Ca^{2+} efflux became stable, 1 nM of PDGF-BB was added to the medium for the first 10 min only. All efflux experiments were performed at room temperature (22° to 24°C).
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25. Confluent cultures of CHO-K1 cells expressing wild-

type PDGF receptor in 100-mm dishes were incubated at 4°C for 2 hours in the presence or absence of 10 nM PDGF-BB before lysis in RIPA buffer [50 mM NaCl, 50 mM NaF, 30 mM sodium pyrophosphate, 5 mM EDTA, 10 mM tris (pH 7.4), 1% Triton X-100, 1 mM PMSF, 25 μM leupeptin, 10 μM pepstatin A, 0.2 U/ml aprotinin and 1 mM sodium vanadate]. The lysates (0.5 ml) were centrifuged at 38,000 rpm at 4°C for 20 hours in a SW 40.1 rotor through 10 ml of a linear gradient of 5 to 20% sucrose in 20 mM Hepes (pH 7.4), 0.15 M NaCl, 2.5 mM $MgCl_2$, 0.6 mM $MnCl_2$, 10% glycerol, 0.2% Triton X-100, 0.02% NaN_3, 0.5 mM vanadate, 1 mM PMSF, and 10 μM leupeptin. After centrifugation, 0.5-ml fractions were collected, precipitated in methanol, and subjected to SDS-PAGE (7%). Alkaline phosphatase (7S) and catalase (11.3S) were used as sedimentation markers.

26. The cells were lysed in RIPA buffer. Insoluble material was removed by centrifugation at 4°C for 10 min at 13,000*g*. The lysates were incubated with Ab 88 at 4°C for 3 hours and the immune complexes were precipitated by protein A–Sepharose beads (Pharmacia). The immunoprecipitates were washed three times with RIPA buffer and three times with buffer containing 0.5 M LiCl and 0.1 M tris (pH 7.4) before analysis by SDS-PAGE.
27. After overnight incubation of the injected oocytes, healthy oocytes were labeled with [^{35}S]methionine (1 mCi/ml, DuPont–New England Nuclear) in MBS (10 μl per oocyte) for 24 hours at 19°C.
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FTZ-F1, a Steroid Hormone Receptor–Like Protein Implicated in the Activation of *fushi tarazu*

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The *Drosophila* homeobox segmentation gene *fushi tarazu* (*ftz*) is expressed in a seven-stripe pattern during early embryogenesis. This characteristic pattern is largely specified by the zebra element located immediately upstream of the *ftz* transcriptional start site. The FTZ-F1 protein, one of multiple DNA binding factors that interacts with the zebra element, is implicated in the activation of *ftz* transcription, especially in stripes 1, 2, 3, and 6. An FTZ-F1 complementary DNA has been cloned by recognition site screening of a *Drosophila* expression library. The identity of the *FTZ-F1* complementary DNA clone was confirmed by immunological cross-reaction with antibodies to FTZ-F1 and by sequence analysis of peptides from purified FTZ-F1 protein. The predicted amino acid sequence of FTZ-F1 revealed that the protein is a member of the nuclear hormone receptor superfamily. This finding raises the possibility that a hormonal ligand affects the expression of a homeobox segmentation gene early in embryonic development.

THE PROCESS OF SEGMENTATION IN the *Drosophila* embryo is governed by a hierarchical network of maternal and zygotic genes (*1*). The *fushi tarazu* (*ftz*) gene is a well-studied zygotic segmentation gene and encodes a protein that can function as a transcription factor through the DNA binding specificity of its homeodomain (*2*). Expression of *ftz* in the even-numbered parasegmental primordia of the embryonic blastoderm (the seven-stripe pattern) is crucial for proper development of the corresponding body segments in the *Drosophila* embryo (*3*).

Expression of *ftz* is controlled primarily at the level of transcription. Sequences that confer the seven-stripe pattern of expression have been localized to ~600 bp of DNA upstream of the *ftz* structural gene (the zebra element) (*4*). Among several transacting factors that bind directly to the zebra element, the FTZ-F1 protein has been implicated as a positive regulator of *ftz* transcription (*5*). Transformed embryos that carry a zebra element–*lacZ* construct mutated at a FTZ-F1 binding site show a pronounced decrease of β-galactosidase activity in the anterior three *ftz* stripes and stripe six, in addition to an overall decrease in activity (*5, 6*).

We purified FTZ-F1 to homogeneity from *Drosophila* embryos and studied the properties of FTZ-F1 protein in detail (*5*). The DNA binding activity of FTZ-F1 is detectable in early (1.5- to 4-hour) embryo extracts, coincident with the expression of *ftz*. An electrophoretically altered form of FTZ-F1 can also be detected at a second, later phase of embryogenesis (after 13 hours). This late activity may be related to the subsequent repression of *ftz* gene or to the regulation of other genes.

In this report, we present the cloning and sequence analysis of *FTZ-F1*. In order to clone *FTZ-F1*, we screened a 0- to 16-hour embryo cDNA expression library with a concatenated FTZ-F1 DNA binding site. Out of 8×10^6 plaques, one interacted with the wild-type FTZ-F1 recognition sequence, but not with a sequence mutated at nucleotides important for FTZ-F1 binding (*5*) (Fig. 1A). Recombinant protein extracted from this clone showed specific binding to the wild-type FTZ-F1 recognition sequence, as analyzed by an electrophoretic mobility shift assay (Fig. 1B, lanes 1 to 4). The recombinant and the natural FTZ-F1 proteins also displayed identical contacts with the recognition sequence, as shown by a methylation interference assay (Fig. 1C). We further tested the recombinant protein for cross-reaction with antibodies to purified FTZ-F1. The antiserum, which inhibits binding of the early and late embryo FTZ-F1 protein to DNA (*5*), inhibited the binding of the recombinant protein (Fig. 1B, lanes 5 to 7).

We isolated overlapping cDNAs for the early form of FTZ-F1 by screening early embryo cDNA libraries with the initial *FTZ-F1* cDNA clone. Sequence analysis of the cDNA clones revealed a continuous open reading frame (ORF) of 1043 amino acids (Fig. 2). The predicted molecular size of the early form of FTZ-F1 is 110 kD, greater than the 95-kD size of late embryo FTZ-F1 as measured by SDS–polyacrylamide gel electrophoresis (*5*). The size difference could be due to modification, anomalous electrophoretic mobility, or intrinsic differences between early and late FTZ-F1 proteins (*5*). Additional evidence that FTZ-F1 is encoded by this ORF was obtained by microsequencing six tryptic peptides derived from FTZ-F1 protein purified to homogeneity from late stage (12- to 24-hour) embryos (2 kg). The sequences of the FTZ-F1 peptides are found without discrepancy in the ORF (underlined residues in Fig. 2). The correspondence of the six peptides derived from late embryo FTZ-F1 with the predicted amino acid sequence of early embryo FTZ-F1 implies that the early and late FTZ-F1 proteins are similar.

A search of the protein sequence database revealed sequence similarity between FTZ-F1 and members of the nuclear hormone receptor superfamily (*7*). The conserved regions include the DNA binding domain, which bears two potential Cys_2-Cys_2 zinc finger motifs and the ligand binding domain of the nuclear receptor superfamily (Fig. 3A). The linear separation between these two domains is greater for FTZ-F1 than for other nuclear receptors. The putative DNA binding domain (region I) of FTZ-F1 is well conserved across the nuclear receptor superfamily, showing identity with all 20 invariant amino acids (*7*). Nonetheless, FTZ-F1 is somewhat distinct from the two major classes of nuclear receptors, which bind to either the glucocorticoid or the estrogen-thyroid hormone response ele-

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